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The Genome of Yoka Poxvirus▽

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Yoka poxvirus was isolated almost four decades ago from a mosquito pool in the Central African Republic. Its classification as a poxvirus is based solely upon the morphology of virions visualized by electron microscopy. Here we describe sequencing of the Yoka poxvirus genome using a combination of Roche/454 and Illumina next-generation sequencing technologies. A single consensus contig of ~175 kb in length that encodes 186 predicted genes was generated. Multiple methods were used to show that Yoka poxvirus is most closely related to viruses in the *Orthopoxvirus* genus, but it is clearly distinct from previously described poxviruses. Collectively, the phylogenetic and genomic sequence analyses suggest that Yoka poxvirus is the prototype member of a new genus in the family *Poxviridae*.

Poxviruses are currently classified into two subfamilies, the *Chordopoxvirinae* (ChPV) and the *Entomopoxivirinae*. The ChPV infect vertebrates, and the *Entomopoxivirinae* infect insects. Within the ChPV subfamily, there are nine genera currently recognized, with classification based initially on morphological and biological characteristics: *Avipoxvirus*, *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Molluscipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *Suipoxvirus*, and *Yatapoxvirus*. More recently, comparative genome analysis has confirmed this poxvirus genus classification (10, 15). The ChPV genomes are composed of linear double-stranded DNA, vary in size from 134 (6) to 365 kb (22), and contain between 130 and 328 open reading frames (ORFs). It has been shown that the ends of the vaccinia virus genome contain covalently closed hairpin loops, indicating that the linear double-stranded DNA molecule consists of a single, continuous polynucleotide chain (4). Typically, terminal inverted repeats are located at the termini of poxvirus genomes. The relatively conserved central region of the ChPV genomes contains essential genes with roles in transcription, replication, and virion assembly and ranges in size from about 80 to 100 kb. The variable genes in poxviruses are located predominantly at either end of the genomes. These genes include some with host range restrictions and immune subversion functions (17), although the functions of many genes in these regions of the poxvirus genomes are not known. Complete genomic sequences of representative viruses from all nine of the ChPV genera have been obtained.

Yoka poxvirus is an unclassified poxvirus that was originally isolated from a pool of mosquitoes (*Aedes simpsoni*) collected in the Central African Republic in 1972 (25). Yoka poxvirus causes the cytopathic effect (CPE) in Vero, CER, PS, and BHK-21 cells but does not form plaques; suckling mice die within 6 days after intracranial inoculation. Virions of poxvirus shape can be observed in the cytoplasm, and the virus does not react serologically with antibodies to variola or vaccinia viruses (25). Based on electron microscopy, Yoka poxvirus was placed in the *Poxviridae* family, and it was suggested to be either an avipox, orthopox, leporipox, goatpox, or sheeppox virus but not a tanapox or a parapox virus (25). Here we report the genome sequence of Yoka poxvirus and provide data suggesting that Yoka poxvirus is the prototype member of a new genus within the family *Poxviridae*.

MATERIALS AND METHODS

Virus strain. The Yoka poxvirus used in this study was strain DakArB 4268, which was isolated from a pool of *Aedes simpsoni* mosquitoes collected in Botambi, Central African Republic, on 15 December 1972. The original virus isolate was obtained by intracerebral inoculation of newborn mice; a sample from the ninth mouse passage level was deposited in the World Reference Center for Emerging Viruses and Arboviruses by J. P. Digoutte, Institute Pasteur, Dakar, Senegal, in 1989. The virus was subsequently inoculated into Vero cell cultures to obtain material for sequencing and for electron microscopy.

Transmission electron microscopy. For ultrastructural analysis, infected Vero cells were fixed for at least 1 h in a mixture of 2.5% formaldehyde prepared from paraformaldehyde powder and 0.1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3) to which 0.03% picric acid and 0.03% $CaCl_2$ were added. The monolayers were washed in 0.1 M cacodylate buffer, and cells were scraped off and processed further as pellets. The pellets were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.3) for 1 h, washed with distilled water, and *en bloc* stained with 2% aqueous uranyl acetate for 20 min at 60°C. The pellets were dehydrated in ethanol, processed through propylene oxide, and embedded in Poly/Bed 812 (Polysciences, Warrington, PA). Ultrathin sections were cut on a Leica EM UC7 ultramicrotome instrument (Leica Microsystems, Bannockburn, IL), strained with lead citrate, and examined in a Philips 201 transmission electron microscope at 60 kV.

Preparation and sequencing of viral DNA. DNA was purified from Yoka-infected Vero cells with a Qiagen DNeasy kit according to the manufacturer's instructions. DNA was sequenced using three methods. (i) Initial sequencing was performed using a sequence-independent PCR amplification strategy followed by 454 genome sequencer (GS) FLX sequencing performed as described previously (9). Sequences were then trimmed to remove primer B sequences prior to assembly using the Newbler program (454 Life Sciences, Branford, CT). (ii) DNA extracted from Vero cell culture was amplified using a Phi29 DNA polymerase-based strategy (Illustra GenomiPhi V2 DNA amplification kit; GE Healthcare, United Kingdom) as described by the manufacturer. Five micro-

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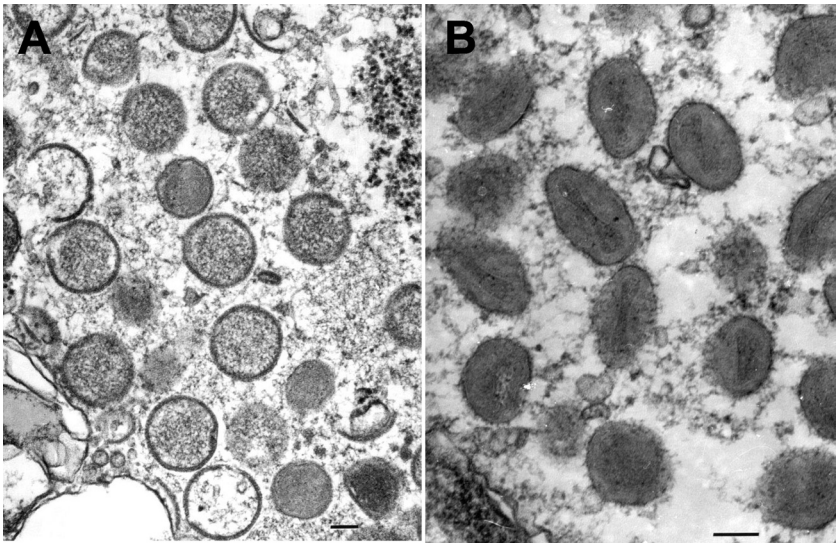

FIG. 1. Ultrastructure of Yoka poxvirus. (A) Different stages of assembly and maturation of virions in the cytoplasm of a Vero cell. (B) Mature Yoka poxvirus virions in the cytoplasm of a Vero cell. A fragment of the host cell nucleus can be seen in the lower left corner. Bars, 100 nm.

grams of the Phi29-amplified DNA was used to construct a library for half-plate sequencing using a 454/Roche GS FLX Titanium system. (iii) Five micrograms of Phi29-amplified products was supplied to Cofactor Genomics (St. Louis, MO) for library preparation and DNA sequencing using the Illumina genome analyzer platform (Illumina, Inc., San Diego, CA).

Sequencing and assembly of the viral genome. Because the DNA used for sequencing contained a mixture of Vero DNA and Yoka poxvirus DNA, we used a customized informatics pipeline as described previously (8), with minor modifications, to computationally distinguish between sequence reads derived from Vero cell DNA and those derived from Yoka poxvirus. Briefly, CD-HIT (14) was used to remove identical and nearly identical sequence reads. Repetitive and low-complexity sequences were masked using RepeatMasker (www.repeatmasker.org/) to generate a high-quality data set. This data set was then sequentially compared with (i) the human genome using BLASTn (3), (ii) GenBank nucleic acid databases using BLASTn (3), and (iii) the NCBI nonredundant (nr) protein database using BLASTx (3) in order to define the origin of each read based on the taxonomy of the top-scoring BLAST hit. Minimal E value cutoffs of 1e−10 and 1e−5 were applied for BLASTn and BLASTx searches, respectively. Sequences identified as viral as well as sequences that had no significant hit to any sequence (50.3% of the unique reads) were assembled using Newbler with default parameters. Illumina data were assembled using Velvet software (26). Contigs generated from both platforms were compared. Illumina data were used to resolve sequences in homopolymeric stretches. Other conflicts between the two assemblers were resolved by targeted PCR and cloning of the amplicon, followed by Sanger sequencing of cloned fragments.

Sequence and phylogenetic analysis of the Yoka poxvirus genome. Sequence analysis and genome annotation were conducted as described for other recently sequenced poxviruses (1, 2, 23). Briefly, ORFs were predicted using FgenesV (SoftBerry, Inc.; www.softberry.com/) and Getorf (Emboss package). All predicted ORFs of more than 90 nucleotides with canonical start and stop codons were identified and aligned to the NCBI nr database using BLAST programs (3). One hundred eighty-six ORFs were annotated as potential genes and numbered from left to right as previously described (1, 2, 22, 23). Multiple sequence alignments were performed with ClustalW (21). Phylogenetic analysis was performed using both maximum likelihood and maximum parsimony methods in the PHYLIP package (7) with 100 bootstrap replicates. Trees with the same topology were generated using both methods for all the data sets used in the study. Phylogenetic trees were visualized using TreeView (16). Tandem Repeats Finder (5) was used to identity tandem repeats in the genome.

Viruses analyzed and sequence accession numbers used for analyses. The viruses analyzed included the following genera, species, and strains: the *Orthopoxvirus* species/strains camelpox virus M-96 (CMLV-M-96; NC_003391.1), camelpox virus CMS (CMLV-CMS; AY009089.1), cowpox virus Brighton red (CPXV; AF482758.2), ectromelia virus (ECTV; AF012825.2), monkeypox virus (MPXV; AF380138.1), vaccinia virus strain Western Reserve (VACV-WR; NC_006998.1), vaccinia virus strain Ankara (VACV-ANK; U94848.1), vaccinia virus strain Copenhagen (VACV-COP; M35027.1), variola virus strain India 1967 (VARV-IND; NC_001611.1), variola virus strain Bangladesh 1975 (VARV-BSH; L22579.1), variola virus strain Garcia 1966 (VARV-GAR; Y16780.1), taterapox virus (TATV; NC_008291.1), horsepox virus (HSPV; ABH08169.1), skunkpox virus (SKPV; AAZ17439.1), volepox virus (VPXV; AAZ17440.1), raccoonpox virus (RCNV; AAZ17441.1), and rabbitpox virus (RPXV; AAS49767.1); the *Parapoxvirus* species bovine papular stomatitis virus (BPSV; AY386265.1), orf virus (ORFV; AY386264.1), and pseudocowpox virus (PCPV; NC_013804.1); the *Avipoxvirus* species canarypox virus (CNPV; AY318871.1) and fowlpox virus (FWPV; AF198100.1); the *Molluscipoxvirus* species molluscum contagiosum virus (MOCV; U60315.1); the *Leporipoxvirus* species myxoma virus (MYXV; AF170726.2) and rabbit fibroma virus (RFV; AF170722.1); the *Suipoxvirus* species swinepox virus (SWPV; AF410153.1); the *Capripoxvirus* species/strains lumpy skin disease virus (LSDV; AF325528.1), sheeppox virus 17077-99 (SPPV; NC_004002.1), and goatpox virus Pellor (GTPV; NC_004003.1); the *Cervidpoxvirus* species/strains deerpox virus W-848-83 (DPV-W83; NC_006966.1) and deerpox virus W-1170-84 (DPV-W84; NC_006967.1); the *Yatapoxvirus* species Yaba-like disease virus (YLDV; NC_002642.1), Yaba monkey tumor virus (YMTV; NC_005179.1), and Tanapox virus (TANV; NC_009888.1); and the unassigned viruses crocodilepox virus (CRV; YP_784249.1) and Yoka poxvirus.

Nucleotide sequence accession number. The sequence of the Yoka poxvirus genome has been deposited in the NCBI database under GenBank accession number HQ849551.

RESULTS AND DISCUSSION

Ultrastructural characteristics. In the cytoplasms of infected Vero cells, structures characteristic for poxvirus assembly and maturation were detected (Fig. 1A), and mature virions with dumbbell-shaped cores (Fig. 1B) were also seen. Their size was ~150 by 250 nm.

Genome sequencing of Yoka poxvirus. Yoka poxvirus was isolated from a mosquito pool in 1972 in the Central African Republic. Electron microscopy suggested that it is a poxvirus (25), but no further characterization has been described in the literature to date. Critically, we are not aware of any molecular data demonstrating that Yoka poxvirus is in fact a poxvirus. To address this issue, we first performed preliminary analysis using a panviral DNA microarray (24), which yielded strong hybridization signals from multiple oligonu-

cleotides derived from a variety of known poxviruses (data not shown). This analysis was followed by partial sequencing by 454 pyrosequencing of the total nucleic acids extracted from lysates of infected Vero cells. While the majority of sequence reads obtained were from the Vero cell host, this effort yielded multiple sequences with significant amino acid sequence similarity (BLAST E values of <9e−07) to ChPV proteins (data not shown).

Based on these initial results, we performed mass sequencing on amplified viral DNA extracted from Vero cell cultures, using both the Roche/454 and Illumina platforms in order to obtain the full genome sequence. From the raw 454 sequencing data (824,955 total raw reads), we assembled four contigs (a total aggregate length of 172,545 nucleotides [nt]) that possessed readily detectable sequence identity to known poxviruses. Approximately 18× coverage of the contigs was achieved. The order of the four contigs was determined by alignment of the contigs to known poxvirus genomes. Sequence assembly from Illumina sequencing data (a total of 13,458,577 60-nt single-end raw reads) resulted in 10 contigs (a total aggregate length of 163,804 nt with an average of ~72× coverage) that possessed detectable sequence identity to known poxviruses. Of the three gaps that separated the four 454-generated contigs, the 3′-most gap was spanned by one Illumina-generated contig, which was confirmed by Sanger sequencing of cloned PCR amplicons covering this region. The other two gaps in the 454-generated data were closed by designing PCR primers to span the gaps and subsequent Sanger sequencing of the cloned PCR amplicons.

Comparison of the independently assembled 454 and Illumina data yielded two classes of sequence discrepancies. There were eight regions in Illumina-assembled contigs with deletions of multiple nucleotides compared to those of the 454 contigs. PCR amplification and Sanger sequencing yielded data consistent with the 454 data in all eight of these cases. In the second category, there were 31 instances of conflicts in homopolymer runs in the well-aligned sequences outside the eight regions with deletions. Because of the known propensity for errors to arise in homopolymers in 454 data, the Illumina consensus sequences were used to correct errors in homopolymer regions. A final consensus contig of 175,699 bp was obtained.

Genome structure of Yoka poxvirus. The poxvirus genome is a linear double-stranded DNA molecule containing inverted terminal repeats of variable size (<0.1 to 12.4 kb) (13). Yoka poxvirus has the same genomic structure, with an inverted terminal repeat of 2.3 kb. No tandem repeats were detected within the inverted terminal repeat region. Because the genomic terminal loops were not sequenced, the left-most nucleotide of the assembled sequence was arbitrarily designated nucleotide number 1, as in other poxviruses (1, 22–23).

The nucleotide composition of the Yoka poxvirus genome was determined to be 74.42% A+T. Previous research has shown that each poxvirus genus has a characteristic A+T content (13). For example, parapoxviruses and molluscipoxviruses have a relatively low A+T content (<40%), orthopoxviruses have an A+T content of approximately 65%, leporipoxviruses have an A+T content of 56 to 60%, and the A+T content of capripoxvirus and suipoxvirus is 72 to 74%. Thus, the A+T content of the Yoka poxvirus genome is most similar to that of the capri- and suipoxviruses (13).

A total of 186 ORFs were annotated as potential genes and numbered from left to right in the Yoka poxvirus genome using the following criteria: they were predicted by FgenesV (SoftBerry, Inc.; www.softberry.com) or Getorf (Emboss package) (1), they shared significant sequence similarity to known viral or cellular genes (BLAST E value of ≤1e−5) (2), or they were 180 nucleotides or longer. A description of all ORFs is given in Table 1. The 186 predicted ORFs represent a 93% coding density, with an average ORF length of 877 nucleotides (encoding proteins of 33 to 1,852 amino acids). There were 10 smaller predicted ORFs that are completely contained within larger ORFs. Amino acid sequence identity between predicted Yoka poxvirus proteins and the vaccinia virus orthologs varied from 22.5 to 92.3%.

Chordopoxvirus core genes. Poxvirus genes can be classified as being core conserved, genus specific, or species specific. There are 90 genes that are involved in key functions such as replication, transcription, and virion assembly that are common to all ChPVs (10). Yoka poxvirus orthologs of all 90 conserved core genes were identified with 37.3 to 92.3% amino acid identity (E value of 3e−8 to 0) to vaccinia virus orthologs. These genes are colinear with those of vaccinia virus (Table 1).

Yoka poxvirus is most closely related to but distinct from orthopoxviruses. To assess the phylogenetic relationship between Yoka poxvirus and other known poxviruses, we performed phylogenetic analysis using representative DNA polymerase protein sequences from all available full-length poxvirus DNA polymerases in the NCBI nr database (as of 25 October 2009). We found that Yoka poxvirus formed a branch separate from those of all other orthopoxviruses (Fig. 2A). Phylogenetic analysis using the DNA-dependent RNA polymerase subunit rpo147 yielded a tree with the same topology (data not shown).

To further assess the phylogenetic relationship of Yoka poxvirus to other poxviruses, we used concatenated amino acid sequences of 35 conserved proteins from each ChPV species as previously described (10). The use of multiple protein sequences for analysis is more robust than single-sequence analysis in constructing phylogenetic trees because more gene loci are sampled and thus the number of phylogenetically informative sites is greatly increased. We found that while Yoka poxvirus was most closely related to viruses in the *Orthopoxvirus* genus (Fig. 2B), it clearly formed a branch separate from those of other orthopoxviruses.

To further define the relationship between Yoka poxvirus and other poxviruses, we also compared the genomic DNA sequences from the central 100-kb region of Yoka poxvirus to those of (i) members of the *Orthopoxvirus* genus and (ii) members of the genera *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Orthopoxvirus*, *Suipoxvirus*, and *Yatapoxvirus*. The 90 core genes of ChPVs are all located within the central ~100-kb region of the genomes. The overall gene order and content are very well conserved among the *Orthopoxvirus*, *Capripoxvirus*, *Leporipoxvirus*, *Suipoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus* genera (10).

Within the *Orthopoxvirus* genus, the central 100-kb region has >90% nucleotide identity among all members, and remarkably, the maximum difference in length for this region is

TABLE 1. Predicted open reading frames of Yoka poxvirus

ORF name	Length (aa)	Genome location (bp)	% amino acid identity	Homolog[a]	Length (aa)	Description/putative function[b]
YKV001	299	1095–199	40	DPV-W83-007	355	IL-1 receptor antagonist
YKV002	224	2159–1488	60	CPXV-BR-209	225	Ortholog of myxoma virus MT-4; ER localization; apoptosis inhibitor
YKV003	182	2930–2385	41	DPV-W83-142	216	C-type lectin-like type II membrane protein
YKV004	1,852	8987–3432	70	CPXV-BR-225	1,919	Surface glycoprotein
YKV005	62	5344–5529	77	CMP202.5L	73	Hypothetical protein
YKV006	127	9520–9140	48	VACWR013 (N/A)	126	Soluble IL-18 binding protein
YKV007	44	9788–9657	80	VACWR018 (N/A)	60	Hypothetical protein
YKV008	229	10021–10707	42	ECTV-MOS-013	241	Zinc finger protein; ubiquitin ligase/host defense modulator
YKV009	65	10868–11062	34	VACWR009 (C11R)	140	Secreted EGF-like protein
YKV010	160	11784–11305	37	CPXV-GER91-020	330	IL-1 receptor antagonist (N terminus only)
YKV011	518	13460–11907	38	VACWR026 (C2L)	512	POZ/BTB kelch domain protein; virulence factor in intradermal mouse model
YKV012	207	14116–13496	53	VACWR027 (C1L)	229	Unknown function
YKV013	120	14514–14155	43	VACWR028 (N1L)	117	Virulence factor; antiapoptotic Bcl-2-like protein
YKV014	208	15165–14542	39	CPXV-BR-010	215	Alpha-amanitin target
YKV015	471	16617–15205	57	VACWR030 (M1L)	472	Ankyrin repeat protein
YKV016	79	16973–16737	31	VACWR034 (K3L)	88	IFN resistance protein
YKV017	96	17302–17015	73	VACWR035 (K4L)	424	Fragment of nick-joining enzyme
YKV018	88	17766–17503	68	VACWR035 (K4L)	424	Fragment of nick-joining enzyme
YKV019	178	18514–17981	28	VACWR040 (F1L)	226	Apoptosis inhibitor (mitochondrial associated)
YKV020	321	19577–18615	87	VACWR043 (F4L)	319	Ribonucleotide reductase, small subunit
YKV021	41	19266–19388	68	m8048R	96	Hypothetical protein
YKV022	73	20726–20508	39	VACWR045 (F6L)	74	Unknown function
YKV023	74	21321–21100	38	VACWR047 (F8L)	65	Unknown function
YKV024	212	22019–21384	76	VACWR048 (F9L)	212	Substrate for poxvirus S-S bond formation pathway
YKV025	438	23322–22009	83	VACWR049 (F10L)	439	Ser/Thr kinase
YKV026	94	23743–23462	38	VACWR050 (F11L)	348	Gene fragment
YKV027	638	26354–24441	56	VACWR051 (F12L)	635	Exclusive to IEV; associates with A36R
YKV028	72	25141–25356	55	VACV-Cop F ORF E	71	Hypothetical protein
YKV029	373	27514–26396	79	VACWR052 (F13L)	372	Palmitoyl protein; major IEV antigen; viral envelopment and egress
YKV030	177	28061–27531				Hypothetical protein
YKV031	49	28240–28094	57	VACWR053.5 (F14.5L)	49	IMV protein; virulence factor
YKV032	148	28713–28270	70	VACWR054 (F15L)	147	Unknown function
YKV033	219	29424–28768	54	VACWR055 (F16L)	231	Unknown function
YKV034	96	29490–29777	75	VACWR056 (F17R)	101	VP11; abundant virion phosphoprotein; required for assembly
YKV035	471	31192–29780	79	VACWR057 (E1L)	479	VP55; poly(A) polymerase catalytic subunit
YKV036	735	33402–31198	67	VACWR058 (E2L)	737	Required for IEV morphogenesis; associates with F12
YKV037	198	34058–33465	42	VACWR059 (E3L)	190	dsRNA-binding protein; inhibits IFN-induced protein kinase PKR
YKV038	255	34835–34071	72	VACWR060 (E4L)	259	DNA-dependent RNA polymerase subunit rpo30
YKV039	384	34876–36027	45	VACWR061 (E5R)	341	Early protein; abundant component of virosome
YKV040	567	36171–37871	78	VACWR062 (E6R)	567	Virion protein; required for assembly
YKV041	270	37877–38686	83	VACWR064 (E8R)	273	Virion core protein; required for transcription in newly infected cells
YKV042	66	38265–38068	77	VACV-Cop E ORF D	66	Hypothetical protein
YKV043	1,008	41720–38697	78	VACWR065 (E9L)	1,006	DNA polymerase
YKV044	96	41752–42039	84	VACWR066 (E10R)	95	Sulfhydryl oxidase
YKV045	129	42435–42049	62	VACWR067 (E11L)	129	Virion core protein
YKV046	656	44392–42425	53	VACWR068 (O1L)	666	Unknown function
YKV047	110	44758–44429	61	VACWR069 (O2L)	108	Nonessential glutaredoxin
YKV048	33	44887–44789	64	VACWR069.5 (N/A)	35	Component of virus entry/fusion complex
YKV049	312	45840–44905	86	VACWR070 (I1L)	312	DNA binding core protein
YKV050	68	46053–45850	64	VACWR071 (I2L)	73	IMV membrane protein
YKV051	266	46854–46057	60	VACWR072 (I3L)	269	ssDNA binding phosphoprotein; associates with ER structures containing viral parental DNA
YKV052	761	49201–46919	79	VACWR073 (I4L)	771	Ribonucleotide reductase, large subunit
YKV053	78	49458–49225	58	VACWR074 (I5L)	79	VP13; IMV protein; nonessential
YKV054	384	50620–49469	72	VACWR075 (I6L)	382	Telomere binding protein; required for virus maturation/encapsidation of DNA
YKV055	422	51881–50616	86	VACWR076 (I7L)	423	Essential viral core cysteine proteinase
YKV056	672	51887–53902	70	VACWR077 (I8R)	676	RNA helicase, DExH-NPH-II domain; required for transcription

Continued on following page

TABLE 1—*Continued*

ORF name	Length (aa)	Genome location (bp)	% amino acid identity	Homolog[a]	Length (aa)	Description/putative function[b]
YKV057	591	55677–53905	72	VACWR078 (G1L)	591	Essential metalloproteinase
YKV058	111	56009–55677	74	VACWR079 (G3L)	111	Essential protein; part of entry/fusion complex
YKV059	220	56003–56662	71	VACWR080 (G2R)	220	Late transcription elongation factor
YKV060	125	57012–56638	67	VACWR081 (G4L)	124	Disulfide oxidoreductase; required for virus maturation and infectivity
YKV061	440	57015–58334	58	VACWR082 (G5R)	434	FEN1-like nuclease; required for homologous recombination and full-size genome formation
YKV062	63	58347–58535	87	VACWR083 (G5.5R)	63	DNA-dependent RNA polymerase subunit rpo7
YKV063	158	58539–59012	66	VACWR084 (G6R)	165	Virulence factor; NlpC/P60 superfamily protein
YKV064	95	58996–59280	62	VACV-Cop G ORF A	132	Hypothetical protein
YKV065	377	60137–59007	66	VACWR085 (G7L)	371	Virion phosphoprotein; required for early morphogenesis
YKV066	260	60168–60947	92	VACWR086 (G8R)	260	Late transcription factor VLTF-1; predicted structural ortholog of PCNA
YKV067	338	60966–61979	71	VACWR087 (G9R)	340	Myristyl protein; part of entry/fusion complex
YKV068	250	61983–62732	83	VACWR088 (L1R)	250	IMV membrane protein; binds to cell surfaces; required for virus entry
YKV069	88	62773–63036	53	VACWR089 (L2R)	87	Formation of crescent membranes and immature virions
YKV070	331	64010–63018	70	VACWR090 (L3L)	350	Virion protein; required for transcription of early genes
YKV071	251	64035–64787	81	VACWR091 (L4R)	251	Abundant virion protein VP8; binds ss/dsDNA
YKV072	128	64805–65188	65	VACWR092 (L5R)	128	Membrane protein; required for cell entry
YKV073	151	65145–65597	74	VACWR093 (J1R)	153	Virion protein; required for assembly
YKV074	177	65620–66150	71	VACWR094 (J2R)	177	Thymidine kinase
YKV075	333	66228–67226	86	VACWR095 (J3R)	333	VP39; poly(A) polymerase subunit; interacts with H4L of rpo complex
YKV076	185	67144–67698	85	VACWR096 (J4R)	185	DNA-dependent RNA polymerase subunit rpo22
YKV077	133	68099–67701	71	VACWR097 (J5L)	133	Late 16-kDa putative membrane protein
YKV078	1,285	68196–72050	88	VACWR098 (J6R)	1,286	DNA-dependent RNA polymerase subunit rpo147
YKV079	171	72565–72053	77	VACWR099 (H1L)	171	Tyr/Ser protein phosphatase; dephosphorylates STAT1
YKV080	224	72486–73157	79	VACWR100 (H2R)	189	Essential component of virus entry complex
YKV081	323	74124–73156	58	VACWR101 (H3L)	324	Immunodominant IMV surface protein; C-terminal transmembrane domain
YKV082	796	76515–74128	83	VACWR102 (H4L)	795	RPO-associated protein RAP94; interacts with VETF
YKV083	186	76695–77252	52	VACWR103 (H5R)	203	Late transcription factor VLTF-4; substrate for B1 kinase; also involved in DNA replication
YKV084	313	77256–78194	77	VACWR104 (H6R)	314	DNA topoisomerase type I
YKV085	144	78218–78649	64	VACWR105 (H7R)	146	Unknown function
YKV086	839	78686–81202	78	VACWR106 (D1R)	844	mRNA capping enzyme, large subunit
YKV087	145	81601–81167	64	VACWR107 (D2L)	146	Virion core protein
YKV088	233	81594–82292	65	VACWR108 (D3R)	237	Virion core protein
YKV089	219	82292–82948	81	VACWR109 (D4R)	218	Uracil-DNA glycosylase; required for DNA polymerase processivity
YKV090	785	82982–85336	82	VACWR110 (D5R)	785	NTPase; contains N-terminal primase and C-terminal superfamily III helicase domains
YKV091	119	84621–84265	69	RFV C6 protein	144	Hypothetical protein
YKV092	636	85369–87276	88	VACWR111 (D6R)	637	Early gene transcription factor; 70-kDa small subunit; VETFS
YKV093	161	87307–87789	85	VACWR112 (D7R)	161	DNA-dependent RNA polymerase subunit rpo18
YKV094	305	88672–87758	44	VACWR113 (D8L)	304	IMV membrane protein; cell surface binding protein; similarity to carbonic anhydrase
YKV095	210	88737–89366	76	VACWR114 (D9R)	213	Nudix family hydrolases; mRNA decapping enzyme
YKV096	259	89366–90142	65	VACWR115 (D10R)	248	Nudix family hydrolases; mRNA decapping enzyme
YKV097	632	92040–90145	84	VACWR116 (D11L)	631	ATPase, nucleoside triphosphate phosphohydrolase I, NPH-I
YKV098	55	91609–91773	63	VACV-Cop D ORF H	61	Similar to YVDH_VACCW; uncharacterized 7.2-kDa protein
YKV099	287	92936–92076	78	VACWR117 (D12L)	287	mRNA capping enzyme, small subunit
YKV100	548	94608–92965	86	VACWR118 (D13L)	551	Rifampin target; trimer; scaffold protein for IMV
YKV101	150	95082–94633	79	VACWR119 (A1L)	150	Late gene transcription factor; VLTF-2
YKV102	224	95775–95104	89	VACWR120 (A2L)	224	Late gene transcription factor; VLTF-3

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TABLE 1—*Continued*

ORF name	Length (aa)	Genome location (bp)	% amino acid identity	Homolog[a]	Length (aa)	Description/putative function[b]
YKV103	76	96002–95775	74	VACWR121 (A2.5L)	76	S-S bond formation pathway; heterodimer with E10R
YKV104	646	97957–96020	81	VACWR122 (A3L)	644	p4b precursor of essential virion protein 4b
YKV105	238	98733–98020	37	VACWR123 (A4L)	281	39-kDa virion core protein; associates with p4a; highly antigenic
YKV106	159	98771–99247	73	VACWR124 (A5R)	164	DNA-dependent RNA polymerase subunit rpo19
YKV107	372	100374–99259	74	VACWR125 (A6L)	372	Virion core protein; required for formation of mature virion
YKV108	711	102546–100414	84	VACWR126 (A7L)	710	Early gene transcription factor; 82-kDa large subunit; VETFL
YKV109	72	101666–101881	74	VACV-Cop A ORF C	128	Overlaps with major ORF
YKV110	287	102596–103456	79	VACWR127 (A8R)	288	Intermediate transcription factor; 32-kDa small subunit; VITF-3
YKV111	93	103733–103455	78	VACWR128 (A9L)	108	Essential in early morphogenesis; associates with viral membranes
YKV112	897	106427–103737	73	VACWR129 (A10L)	891	Precursor p4a of essential virion protein 4a
YKV113	65	103984–104178	74	CMP126.5aR (A orf E)	166	Similar to camelpox virus CMS CMP126.5aR
YKV114	316	106442–107389	84	VACWR130 (A11R)	318	Nonstructural protein; required for formation of virion membrane
YKV115	177	107929–107399	62	VACWR131 (A12L)	192	Virion core protein; cleaved by I7L proteinase
YKV116	61	108138–107956	46	VACWR132 (A13L)	70	Essential IMV membrane protein; phosphorylated; early virion biogenesis
YKV117	91	108414–108142	60	VACWR133 (A14L)	90	Essential IMV membrane protein; phosphorylated
YKV118	53	108592–108434	77	VACWR134 (A14.5L)	53	Nonessential IMV membrane protein; virulence factor in mice
YKV119	94	108866–108585	78	VACWR135 (A15L)	94	Core protein
YKV120	375	109977–108853	58	VACWR136 (A16L)	377	Myristyl protein; cysteine rich; required for entry/fusion
YKV121	212	110590–109955	60	VACWR137 (A17L)	203	IMV membrane protein; complexes with A27/A14; membrane cell fusion activity
YKV122	479	110605–112041	68	VACWR138 (A18R)	493	DNA helicase; transcript release factor
YKV123	73	112246–112028	70	VACWR139 (A19L)	77	Similar to zinc finger protein
YKV124	116	112600–112253	71	VACWR140 (A21L)	117	IMV membrane protein; required for cell entry/fusion
YKV125	425	112599–113873	72	VACWR141 (A20R)	426	Viral DNA polymerase processivity factor
YKV126	188	113809–114372	81	VACWR142 (A22R)	187	Holliday junction resolvase; cleaves replicated concatemers
YKV127	383	114399–115547	77	VACWR143 (A23R)	382	Intermediate transcription factor; 45-kDa large subunit; VITF-3
YKV128	1,166	115547–119044	91	VACWR144 (A24R)	1,164	DNA-dependent RNA polymerase subunit rpo132
YKV129	660	121153–119174	42	VACWR148 (N/A)	725	N-term half of cowpox virus A-type inclusion protein
YKV130	513	122757–121219	40	VACWR149 (A26L)	500	p4c precursor
YKV131	102	123105–122800	53	VACWR150 (A27L)	110	IMV surface protein; protective antigen; binds heparin
YKV132	145	123543–123109	75	VACWR151 (A28L)	146	Virion surface protein; required for cell entry; N-term hydrophobic anchor
YKV133	302	124452–123547	74	VACWR152 (A29L)	305	DNA-dependent RNA polymerase subunit rpo35
YKV134	77	124654–124424	66	VACWR153 (A30L)	77	IMV protein; interacts with F10/G7; required for assembly
YKV135	44	124798–124667	44	VACWR153.5 (A30.5L)	39	Hypothetical protein
YKV136	134	124808–125209	55	VACWR154 (A31R)	124	Unknown function
YKV137	271	125988–125176	75	VACWR155 (A32L)	270	Virion protein; required for assembly; NTP motif
YKV138	48	125426–125569	64	A ORF L	88	Hypothetical protein
YKV139	181	126091–126633	47	VACWR156 (A33R)	185	EEV membrane phosphoglycoprotein; C-type lectin fold; involved in cell-cell spread
YKV140	168	126660–127163	68	VACWR157 (A34R)	168	IEV and EEV membrane glycoprotein; interacts with B5 for intracellular virus trafficking
YKV141	175	127191–127715	52	VACWR158 (A35R)	176	Inhibits MHC class II antigen presentation; virulence factor
YKV142	160	127762–128241	34	VACWR159 (A36R)	221	IEV transmembrane phosphoprotein; interacts with F12; functions in viral egress
YKV143	256	128312–129079	43	VACWR160 (A37R)	263	Unknown function
YKV144	275	130069–129245	68	VACWR162 (A38L)	277	Integral membrane protein; deletion causes reduced plaque size
YKV145	135	130195–130599	57	VACWR167 (A42R)	133	Profilin-like protein; localizes to A type inclusions

Continued on following page

TABLE 1—*Continued*

ORF name	Length (aa)	Genome location (bp)	% amino acid identity	Homolog[a]	Length (aa)	Description/putative function[b]
YKV146	194	130630–131211	28	VACWR168 (A43R)	194	Type I membrane glycoprotein; important for intradermal lesion formation
YKV147	62	131232–131417	62	VACWR169 (N/A)	78	Unknown function
YKV148	316	131457–132404	38	Alpha-2,6 sialyltransferase	413	Similar to avian/mammalian beta-galactoside alpha-2,6-sialyltransferases
YKV149	359	133504–132428	60	VACWR170 (A44L)	346	Hydroxysteroid dehydrogenase; inhibits inflammatory response
YKV150	111	133522–133854	56	VACWR171 (A45R)	125	Inactive Cu-Zn superoxide dismutase-like virion protein; not required for replication
YKV151	249	134784–134038	40	VACWR173 (A47L)	252	Unknown function; immunoprevalent protein
YKV152	207	134900–135520	58	VACWR174 (A48R)	227	Thymidylate kinase
YKV153	159	135533–136009	37	VACWR175 (A49R)	162	Unknown function
YKV154	551	136034–137686	71	VACWR176 (A50R)	552	ATP-dependent DNA ligase
YKV155	333	137720–138718	42	VACWR177 (A51R)	334	Unknown function
YKV156	184	138809–139360	57	VACWR178 (A52R)	190	Toll/IL-receptor-like protein; blocks activation of NF-κB; virulence factor
YKV157	382	139477–140622	31	Ornithine decarboxylase	445	Similar to host ODCs
YKV158	573	140666–142384	42	VACWR180 (A55R)	564	BTB kelch domain protein; altered CPE
YKV159	301	142768–143670	67	VACWR183 (B1R)	300	Ser/Thr kinase; required for DNA replication; downregulates p53 by hyperphosphorylation
YKV160	203	143715–144323	53	VACWR184 (B2R)	219	N-term half of schlafen homolog
YKV161	323	144388–145356	41	VACWR187 (B5R)	317	EEV type I membrane glycoprotein; protective antigen; virulence protein
YKV162	179	145465–146001	27	VACWR188 (B6R)	173	Ankyrin-like protein
YKV163	272	146028–146843	45	VACWR190 (B8R)	272	Soluble IFN-γ receptor-like protein
YKV164	289	147029–147895	50	VACWR194 (B12R)	283	Ser/Thr protein kinase-like protein
YKV165	353	147898–148956	47	VACWR195 (B13R)	345	SPI-2/CrmA; inhibits Fas-mediated apoptosis, IL-1 convertase, lipoxygenase pathway
YKV166	153	149007–149465	57	VACWR196 (B15R)	149	Unknown function
YKV167	351	149727–150779	33	VACWR200 (B19R)	351	IFN-α/β receptor-like secreted glycoprotein
YKV168	595	150838–152622	36	CPXV-BR-221	557	Kelch-like protein
YKV169	357	152650–153699	31	VACWR032 (K1L)	284	Ankyrin-like protein/NF-κB inhibitor
YKV170	221	153741–154403	60	VACWR031 (M2L)	220	NF-κB inhibitor
YKV171	153	154454–154912	59	VACWR021 (C7L)	150	Host range protein; inhibits antiviral activities induced by type I IFNs
YKV172	543	155502–157130	37	VACWR019 (C9L)	634	Ankyrin-like protein (N-terminal deletion)
YKV173	345	157225–158259	23	MHC class I	362	Similar to host MHC class I proteins
YKV174	119	158436–158792	56	CPXV-BR-015	110	vCD30; Cys-rich soluble TNFR-like protein
YKV175	223	158826–159494	35	VACWR031 (M2L)	220	ER localized; inhibits NF-κB activation
YKV176	744	159552–161783	29	CPXV-BR-019	796	Low similarity; ankyrin-like protein
YKV177	352	161847–162902	48	VACWR205 (C12L)	353	Serine protease inhibitor-like protein (SPI-1)
YKV178	127	163062–163442	48	CPXV-BR-227	320	N-term fragment of TNF receptor-like protein (CrmD)
YKV179	479	164056–165492	59	CPXV-BR-231	619	Ankyrin-like protein; truncated at C terminal
YKV180	78	165557–165790	58	CPXV-BR-231	619	C-terminal fragment of large ankyrin-like protein
YKV181	135	165853–166278	48	CPXV-BR-232	355	N-terminal fragment of TNF receptor-like protein (CrmB)
YKV182	195	167293–167877	47	VACWR205 (C12L)	353	SPI-1; active against cathepsin G; host range
YKV183	603	170158–168350	37	VACWR199 (B18R)	574	Ankyrin-like protein
YKV184	806	170970–173387	22	CPXV-BR-019	574	Ankyrin-like protein
YKV185	224	173541–174212	60	CPXV-BR-209	225	Virulence factor
YKV186	299	174605–175501	40	DPV-W83-007	355	IL-1 receptor antagonist

[a] CMP, camelpox; N/A, not available. The gene name of the vaccinia virus homolog is given in parenthesis.

[b] IL-1, interleukin-1; ER, endoplasmic reticulum; EGF, epidermal growth factor; IFN-γ, gamma interferon; IEV, intracellular enveloped virus; IMV, intracellular mature virion; ssDNA, single-stranded DNA; VLTF-1, late transcription factor; VETF, early gene transcription factor; NTPase, nucleoside triphosphatase; NPH-I, nucleoside triphosphate phosphohydrolase 1; VETFL, early gene transcription factor large subunit; VITF, intermediate transcription factor; EEV, external enveloped virion; BTB, bric-a-brac/poxvirus and zinc finger (BTB/POZ) domains; TNFR, tumor necrosis factor receptor; TNF, tumor necrosis factor.

382 nt between the longest and the shortest (10). We found the central region of Yoka poxvirus was 967- to 1,365-bp shorter than that found in the other orthopoxviruses. In addition, Yoka poxvirus shared only 72.4% identity with CPXV, its closest relative in the genus.

We constructed a distance matrix using the sequence alignments of the central 100-kb region of representative viruses from the *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Orthopoxvirus*, *Suipoxvirus*, and *Yatapoxvirus* genera (Table 2). The *Avipoxvirus*, *Molluscipoxvirus*, and *Parapoxvirus* genera were excluded from the analysis because their genomes are too diverged to align properly. The genome of deerpox virus, the type species of the recently added genus *Cervidpoxvirus*, has a genetic distance of 0.3453 to 0.5173

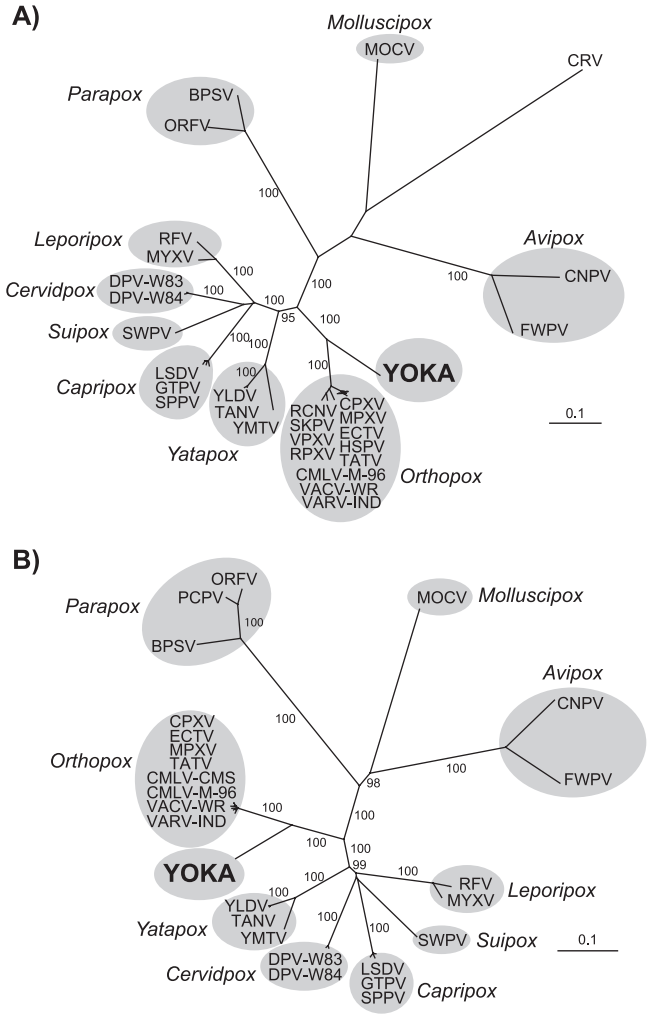

FIG. 2. Phylogenetic analysis of Yoka poxvirus. (A) Phylogenetic tree comparing YKV043 to representative sequences of all full-length poxvirus DNA polymerases in the GenBank database as of 25 October 2009. (B) Phylogenetic analysis of the concatenated amino acid sequences of the core 35 ORFs from all species listed in the *Chordopoxvirinae* subfamily in the ICTV master species list (version 9, 2009) with completely sequenced genomes, as well as the DPV-W84, YLDV, and CMLV-CMS genomes. Multiple sequence alignments were generated using ClustalW. The PHYLIP package (7) was used to generate maximum parsimony phylogenetic trees, and bootstrap values (>90) from 100 replicates are shown.

from all other genera. We found that Yoka poxvirus has a genetic distance of 0.3214 to 0.6009 from all other genera, again suggesting that Yoka poxvirus belongs to a distinct genus.

Novel ORFs that are not present in other poxviruses. Interestingly, Yoka poxvirus also encodes predicted proteins that are more closely related to proteins of vertebrates or *Trypanosoma brucei* than to other poxvirus proteins.

Yoka poxvirus YKV157 shares approximately 30% amino acid identity (BLASTp E value of 4e−47) with a series of ornithine decarboxylase (ODC) proteins, including those from trypanosomes, fish, birds, and mammals. ODC catalyzes the rate-limiting step of polyamine biosynthesis by converting ornithine to putrescine. ODC is the key enzyme in the pathway leading to biosynthesis of polyamines, which are essential for cellular growth and proliferation. Consequently, the polyamine biosynthetic pathway has been a target for the development of agents that prevent carcinogenesis and tumor growth as well as for parasitic treatment (12, 20). Among previously sequenced viruses, only members of the family *Phycodnaviridae* encode proteins that share detectable similarity with YKV157. The most closely related viral sequence, protein NY2A_B278R (ABT14677.1) from *Paramecium bursaria* chlorella virus NY2A, shares 27% amino acid identity (BLASTp E value of 5e−29) with YKV157. Currently, no sequenced poxvirus is predicted to encode an ODC homolog. Multiple sequence alignments amongYKV157, homologs encoded by members of the *Phycodnaviridae*, and mouse, human, and *T. brucei* ODCs suggest that YKV157 has different residues in the substrate binding site as well as in the enzymatic active site. In the phycodnavirus-encoded enzymes, a few amino acid changes in the substrate binding sites change the substrate preference from L-ornithine to L-arginine (18). It is not clear from the sequence alignment alone whether YKV157 possesses similar enzymatic function or which substrates it may act upon. The roles of the arginine decarboxylases of the *Phycodnaviridae* and Yoka poxvirus YKV157 in their viral life cycles remain unclear, although they may affect host protein translation (19).

YKV173 encodes a predicted major histocompatibility complex class I (MHC class I) antigen protein that is more closely related to those of vertebrates than to those of other viral proteins. YKV173 shares 24% identity over 314 amino acids (BLASTp E value of 7e−16) to a mouse MHC class I protein. While a few poxviruses, such as squirrel parapoxvirus, deerpox virus, and molluscum contagiosum virus, encode MHC class I homologs, no significant sequence similarity was detected amongYKV173 and these poxvirus proteins. The N-linked glycosylation site seen in all MHC class I α1 domains is conserved in YKV173. However, multiple sequence alignment of YKV173 with the mouse and the human MHC class I proteins demonstrated that the residues that contribute to peptide binding in the A and F pockets are not well conserved in YKV173. In YKV173, the amino acids at those loci are more similar to residues found in nonclassical MHC class I proteins (11) (data not shown).

These data demonstrate that Yoka poxvirus carries unique ORFs that are not shared with other poxviruses, suggesting that Yoka poxvirus may have evolved under selective pressures that are distinct from those of all other known poxviruses. The natural host of Yoka poxvirus is currently unknown. However, the isolation of Yoka poxvirus from a mosquito pool most likely reflects the presence of infectious virus in the blood meal of the mosquito. Based on phylogenetic analysis, Yoka poxvirus is most closely related to other poxviruses of mammals, suggesting that the natural host of Yoka poxvirus is most likely a mammal.

Our aggregate genome analysis suggests that Yoka poxvirus is the prototype species of a new genus of poxvirus. This conclusion is based on (i) the A+T content of the genome, (ii) the size and degree of sequence conservation of the central ~100-kb region of Yoka poxvirus compared to those of other poxviruses, and (iii) the genetic distance between Yoka poxvirus and poxviruses of other genera. Therefore, we propose the

TABLE 2. Matrix of DNA distances between Yoka poxvirus and viruses of the *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Orthopoxvirus*, *Suipoxvirus*, and *Yatapoxvirus* genera[a]

Genus	Species	DNA distance to indicated genera and species											
		YKV	*Orthopoxvirus* VACV-WR	*Suipoxvirus* SWPV	*Capripoxvirus*			*Cervidpoxvirus* DPV-W83	*Yatapoxvirus*			*Leporipoxvirus*	
					GTPV	LSDV	SPPV		TANV	YLDV	YMTV	MYXV	RFV
Centapoxvirus[b]	YKV												
Orthopoxvirus	VACV-WR	0.3214											
Suipoxvirus	SWPV	0.4725	0.5008										
Capripoxvirus	GTPV	0.4937	0.5284	0.3823									
	LSDV	0.4956	0.5287	0.3829	0.0178								
	SPPV	0.4919	0.5272	0.3810	0.0243	0.0198							
Cervidpoxvirus	DPV-W83	0.4874	0.5173	0.3822	0.3469	0.3479	0.3453						
Yatapoxvirus	TANV	0.4908	0.5154	0.4255	0.4009	0.4026	0.3995	0.3921					
	YLDV	0.4912	0.5151	0.4248	0.3999	0.4014	0.3986	0.3916	0.0117				
	YMTV	0.5108	0.5184	0.4367	0.4160	0.4163	0.4146	0.4068	0.1872	0.1868			
Leporipoxvirus	MYXV	0.6009	0.5702	0.4758	0.4873	0.4850	0.4878	0.4800	0.5058	0.5055	0.4977		
	RFV	0.5839	0.5662	0.4634	0.4688	0.4679	0.4691	0.4639	0.4907	0.4905	0.4878	0.1211	

[a] YKV, Yoka poxvirus.
[b] Proposed name of new genus.

new genus name Centapoxvirus (based on its isolation from the *Cen*tral *A*frican Republic).

Conclusions. In this work, we report the genomic sequence of the Yoka poxvirus and unambiguously define by molecular criteria that it is a member of the family *Poxviridae*. As with all poxviruses, the genome of Yoka poxvirus consists of a central core of conserved genes flanked by more-variable terminal regions. We presented multiple lines of evidence that suggest that Yoka poxvirus defines a novel genus in the family *Poxviridae*. Moreover, Yoka poxvirus carries many highly divergent homologs of poxvirus immunomodulatory genes and accessory genes (Table 1) as well as novel ORFs that are not described for any other poxviruses. The fact that Yoka poxvirus can be cultured in several cell lines, can cause CPE in some cell lines, and can grow in mice makes Yoka poxvirus an attractive new model for studying *in vivo* poxvirus infections and pathogenesis. Moreover, the extensive evolutionary divergence of Yoka poxvirus from other orthopoxviruses provides a unique opportunity to define sequence-function differences among orthologous genes.

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